Acute effects of elevated NEFA on vascular function: a comparison of SFA and MUFA

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Abstract

There is emerging evidence to show that high levels of NEFA contribute to endothelial dysfunction and impaired insulin sensitivity. However, the impact of NEFA composition remains unclear. A total of ten healthy men consumed test drinks containing 50 g of palm stearin (rich in SFA) or high-oleic sunflower oil (rich in MUFA) on separate occasions; a third day included no fat as a control. The fats were emulsified into chocolate drinks and given as a bolus (approximately 10 g fat) at baseline followed by smaller amounts (approximately 3 g fat) every 30 min throughout the 6 h study day. An intravenous heparin infusion was initiated 2 h after the bolus, which resulted in a three- to fourfold increase in circulating NEFA level from baseline. Mean arterial stiffness as measured by digital volume pulse was higher during the consumption of SFA ($P < 0.001$) but not MUFA ($P = 0.089$) compared with the control. Overall insulin and gastric inhibitory peptide response was greater during the consumption of both fats compared with the control ($P < 0.001$); there was a second insulin peak in response to MUFA unlike SFA. Consumption of SFA resulted in higher levels of soluble intercellular adhesion molecule-1 (sI-CAM) at 330 min than that of MUFA or control ($P \leq 0.048$). There was no effect of the test drinks on glucose, total nitrite, plasminogen activator inhibitor-1 or endothelin-1 concentrations. The present study indicates a potential negative impact of elevated NEFA derived from the consumption of SFA on arterial stiffness and sI-CAM levels. More studies are needed to fully investigate the impact of NEFA composition on risk factors for CVD.

Key words: NEFA: Incretin: Insulin: Digital volume pulse

Insulin-resistant states such as obesity and type 2 diabetes (T2D) are characterised by metabolic anomalies such as elevated plasma NEFA levels. Epidemiological studies have shown that elevated plasma NEFA are associated with a greater risk of developing impaired glucose tolerance[1] and T2D[2]. Experimental elevation of plasma NEFA using intravenous infusion of lipid and heparin also results in impaired insulin-stimulated glucose uptake in skeletal muscle[3] and endothelial dysfunction[4], both of which are key features of T2D and obesity[5,6]. There is mounting evidence to suggest that the saturation of dietary fat can influence endothelial function; for example, consumption of SFA during weight-loss regimens has been shown to be negatively associated with flow-mediated dilatation (FMD)[7]. Levels of SFA in serum have also been shown to be inversely correlated with endothelial-dependent forearm blood flow[8,9]. Findings from chronic dietary studies comparing diets rich in SFA or MUFA are mixed. Some short-term studies have found that a SFA-rich diet impairs vascular reactivity compared with a MUFA-rich diet[10] or a diet rich in both MUFA and PUFA[11]. However, an 8-month supplementation trial found no difference in endothelial-dependent or -independent vasodilation measures between MUFA- or SFA-based oils[12]. Similarly, there are contrasting findings from postprandial studies comparing high-fat meals rich in either SFA or MUFA, with some studies finding no differential effect on FMD[13,14] and one study reporting a negative impact of a MUFA-rich meal on FMD[15].

Dietary fat composition may affect endothelial function by modulating insulin sensitivity since the endothelium is an insulin-dependent tissue, and impaired endothelial function is a key feature of T2D and other insulin-resistant states. Although the impact of dietary fatty acid composition on insulin sensitivity remains equivocal[16], one large randomised controlled trial found a decrease in insulin sensitivity after substitution of monounsaturated fat for saturated fat over a 12-week period when total fat consumed constituted less than 37 % energy[17]. Furthermore, the Atherosclerosis Risk in Community Study found that the percentage of SFA in

plasma cholesteryl esters and phospholipids was positively associated with the development of diabetes[18].

Mechanisms that might mediate the variable effects of dietary fat on insulin sensitivity and endothelial function include potentially direct effects of fatty acids on insulin signalling pathways in endothelial cells, as has been described for skeletal muscle[3]. In addition, there are potentially indirect effects of different fatty acids on insulin secretion. The possibility that this might be mediated via an effect on the secretion of incretin hormones (gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1)) is of particular interest given the emerging therapeutic role of GLP-1, in particular in the treatment of T2D. Indeed, infusion of GLP-1 has been shown to cause a marked increase in FMD in T2D patients with stable coronary artery disease[19]. Whether such benefits in incretin secretion might be achieved by dietary changes is unclear, but postprandial studies have indicated that consumption of MUFA-rich meals induces a higher incretin response than consumption of SFA-rich meals[20,21]. Furthermore, Southern European subjects whose habitual diets were rich in MUFA have been shown to have fasting levels of insulin twofold higher and fasting levels of GIP over fourfold higher than a matched group of Northern European subjects[22].

In addition to effects on insulin secretion, fatty acids may have a direct impact on the insulin signalling pathway, influencing the balance between vasodilator and vasoconstrictor responses. Insulin signalling through the phosphoinositide-3 kinase pathway regulates the production of the vasodilator NO, an important regulator of vascular tone[23]. However, an alternative pathway of insulin signalling, the mitogen-activated protein kinase pathway, can lead to the generation of the vasoconstrictor endothelin-1 (ET-1), as well as cellular adhesion molecules such as soluble intercellular adhesion molecule-1 (sI-CAM) and plasminogen activator inhibitor-1 (PAI-1). Evidence from cell culture studies suggests that different fatty acids may affect the balance of these two pathways, with particularly adverse effects of SFA, which may have significance for impaired endothelial function and atherosclerosis[24,25].

Previous experimental studies of acute NEFA elevation have been unable to study variable effects of fatty acid composition since they have mostly used intravenous lipid emulsions such as Intralipid, which are rich in *n*-6 PUFA. The present study aimed to investigate the effects of acute elevation of plasma NEFA of different composition using a relatively new protocol developed by Beysen *et al.*[26], which involves frequent ingestion of fat administered orally rather than by intravenous infusion. We have examined the effect of elevating NEFA derived from consumption of SFA- and MUFA-rich drinks on the levels of insulin and incretin hormones, as well as on circulating measures of endothelial function (total nitrite (NO_x), sI-CAM, ET-1 and PAI-1). In addition, we used digital volume pulse to assess arterial stiffness as a further marker of endothelial function.

Experimental methods

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Reading Ethics Committee. Written informed consent was obtained from all subjects. The study was conducted in the Hugh Sinclair Unit of Human Nutrition at the University of Reading.

The method of elevating NEFA of specific types is based on that by Beysen *et al.*[27]. A drink mixture was prepared comprising 50 g of either palm stearin (SFA; 59 % 16 : 0, 28 % 18 : 1*n*-9, 6 % 18 : 2*n*-6, 5 % 18 : 0) or high-oleic sunflower oil (MUFA; 81 % 18 : 1*n*-9, 10 % 18 : 2*n*-6, 4 % 16 : 0, 3 % 18 : 0), 30 g skimmed milk powder (Premier International Foods Limited, Birmingham, UK), 15 g chocolate powder (The Spanish Chocolate Company Limited, Leicester, UK) and 0·5 g monoacylglycerol emulsifier (Danisco, Copenhagen, Denmark). Water was added to achieve a final weight of 320 g, and the mixture was blended for several minutes to ensure emulsification. The test drinks were identical in protein (11·5 g) and carbohydrate (28·1 g) content. The drinks were divided into a main test drink of 62 g (approximately 10 g fat, 5·4 g carbohydrate and 2·2 g protein) and twelve smaller drinks of 21 g (approximately 3 g fat, 1·8 g carbohydrate and 0·75 g protein). Control drinks were prepared using the same method but excluding the oils. All drinks were served warm to enhance palatability.

Healthy men (*n* 10; Table 1) were recruited to take part in three study days. All subjects were non-smokers of normal body weight and were not suffering from hyperlipidaemia or any endocrine disorder likely to affect lipid metabolism; all showed normal lipid and standard clinical biochemistry values on screening. Subjects attended the Unit on three occasions separated by at least 1 week and were randomly assigned to one of the test drinks on each day. For 24 h before each study day, subjects were asked to refrain from strenuous exercise and alcohol and were supplied with a low-fat ready meal to be consumed in the evening. On each study day, subjects arrived after an overnight fast, and an intravenous cannula was placed at the wrist of the non-dominant arm. The main test drink was consumed at 0 min, with the smaller drinks given every 30 min for a further 6 h. At 120 min, a second cannula was inserted into the antecubital vein in the non-dominant arm for the infusion of heparin. A bolus of heparin (500 IU) was administered, followed by a

Table 1. Baseline characteristics of the ten male subjects

(Mean values and standard deviations)

	Mean	SD
Age (years)	24·8	7·2
Ht (m)	1·8	0·1
Wt (kg)	74·2	10·2
BMI (kg/m²)	22·5	2·4
Waist circumference (cm)	84·1	5·4
BP (mmHg)		
Systolic	128	10
Diastolic	74	5
Fasting total cholesterol (mmol/l)	4·1	0·9
Fasting glucose (mmol/l)	5·5	0·6
Fasting TAG (mmol/l)	0·9	0·3

BP, blood pressure.

continuous infusion of heparin (0·4 IU/kg body weight per min) for the rest of the study day.

Stiffness index derived from digital volume pulse (SI_{DVP}) was measured four times during each study day using digital volume pulse with a PulseTrace PCA 2 (Micro Medical Limited, Kent, UK). SI_{DVP} has been shown to be positively correlated with classical CVD risk factors such as age, waist:hip ratio, blood pressure and carotid intima-media thickness[28]. The PulseTrace equipment calculates SI_{DVP} by recording three consecutive waveforms through the index finger pulse; SI_{DVP} is calculated by dividing the subject's height by the difference in time between the systolic-derived peak and the diastolic-derived peak of the waveform. In addition to the replicates performed by PulseTrace, this technique was performed three times, giving an average of nine readings at each time point.

Blood samples were collected every 30 min into K_3EDTA or serum tubes and centrifuged at 4°C within 30 min of collection. For analysis of GIP, 10 µl of dipeptidyl peptidase IV inhibitor (Millipore Corporation, Watford, Herts, UK) per ml of blood were added at the time of collection to protect against proteolysis. Samples were stored immediately at −20°C until analysis. Serum NEFA were quantified using an automated clinical chemistry analyser (ILAB 600) with kits supplied by Alpha Laboratories (Eastleigh, Hants, UK). Instrumentation Laboratories (Warrington, Ches, UK) supplied kits for the analysis of serum TAG, glucose and total cholesterol. Serum insulin was measured using a dissociation-enhanced lanthanide fluorescent immunoassay kit (Perkin Elmer Limited, Beaconsfield, Bucks, UK). Plasma GIP and serum C-peptide were measured using commercially available colorimetric ELISA kits (Millipore Corporation, Watford, Herts, UK) as were serum sI-CAM, plasma PAI-I and ET-1 (R&D Systems Europe Limited, Abingdon, Oxon, UK). Total serum nitrites (NO_x) were measured using a NO quantification kit (Actif Motif, Rixensart, Belgium).

In four subjects, fatty acid composition analysis of NEFA and TAG was performed on baseline fasted samples and those collected between 300 and 360 min following the start of the oral fat load. Lipids were extracted from 800 µl serum using chloroform–methanol (2:1, by vol.) containing butylated hydroxytoluene (50 mg/ml) before being applied to a solid-phase extraction cartridge (Varian, Oxford, Oxon, UK) to isolate the NEFA and TAG fractions. These fractions were then saponified and methylated in methanol containing 2 % (v/v) H_2SO_4 at 70°C for 1 h. Fatty acid methyl esters were recovered by extraction into hexane and analysed in a gas chromatograph as described previously[29]. Fatty acid methyl esters were identified by comparison of retention times against a known standard, Supelco 37 component fatty acid methyl esters mix (Supelco, Dorset, UK).

Statistical analysis

All statistical analyses were performed using SPSS software (version 15.0; SPSS, Inc., Chicago, IL, USA). Data were tested for normality using the Shapiro–Wilk test and log-transformed if necessary. Analytes measured at least four times a day (NEFA, TAG, glucose, insulin, C-peptide and GIP) were compared using repeated-measures ANOVA. This approach was also used to compare the fatty acid composition of NEFA and TAG between the study days. Analytes measured only at 0 and 330 min (sI-CAM, NO_x, ET-1 and PAI-1) were analysed with repeated-measures ANCOVA using 330 min as the dependent variable and 0 min as a covariate. In both cases, a Bonferroni correction was applied to control for multiple comparisons. Values of $P \leq 0.05$ were considered as significant.

Results

Baseline subject characteristics are shown in Table 1. There were no significant differences in any baseline measures between the test drinks.

Serum NEFA, TAG and glucose

There was an initial decline in NEFA after consumption of the bolus oral load followed by a sharp increase at 120 min following initiation of heparin infusion. The oral fat–heparin protocol resulted in a threefold elevation of serum NEFA following consumption of both fats compared with that of the control (Fig. 1). Repeated-measures ANOVA revealed a significant effect of test drink and time ($P < 0.001$); both MUFA- and SFA-rich drinks ($P < 0.001$) elicited a higher NEFA response than the control, with no significant difference between the two fats ($P = 0.193$).

The fatty acid composition of NEFA and TAG at baseline ($t = 0$ min) and after consumption of the control, SFA and MUFA-rich drinks ($t = 300–360$ min) is shown in Table 2. A significant effect of test drink was found for palmitic acid (NEFA and TAG, $P < 0.001$), stearic acid (NEFA only, $P = 0.001$), oleic acid (NEFA and TAG, $P \leq 0.001$) and linoleic acid (NEFA $P = 0.001$ and TAG $P = 0.020$). There was an approximately 1·5-fold increase in the palmitic acid content of both NEFA and TAG following consumption of the SFA test drinks. Consumption of the MUFA test drinks led to an approximately 2·5-fold increase of oleic acid in NEFA and an approximately 1·7-fold increase in TAG, which was accompanied by significant decreases in palmitic acid. Consumption of the control drinks did not affect proportions of any of the fatty acids from baseline in either NEFA or TAG.

A significant effect of test drink, time and test drink × time interaction (all $P < 0.001$) was found for serum TAG (Fig. 1(b)). Consumption of both MUFA- and SFA-rich drinks ($P < 0.001$) was followed by a rise in serum TAG with no rise following consumption of the control drink. There was an initial decline in serum TAG following the administration of heparin at 120 min, but thereafter, values remained constant. There was no significant difference in the serum TAG profiles for the two test fats ($P = 1.000$).

Serum glucose levels initially increased at 30 min following the bolus oral load on all three drinks and then declined to below-fasting levels until 240 min when there was a steady increase, most markedly for the two fat test drinks following heparin infusion (Fig. 2(c)). There was a significant effect of time ($P < 0.001$) but not of test drink ($P = 0.118$).
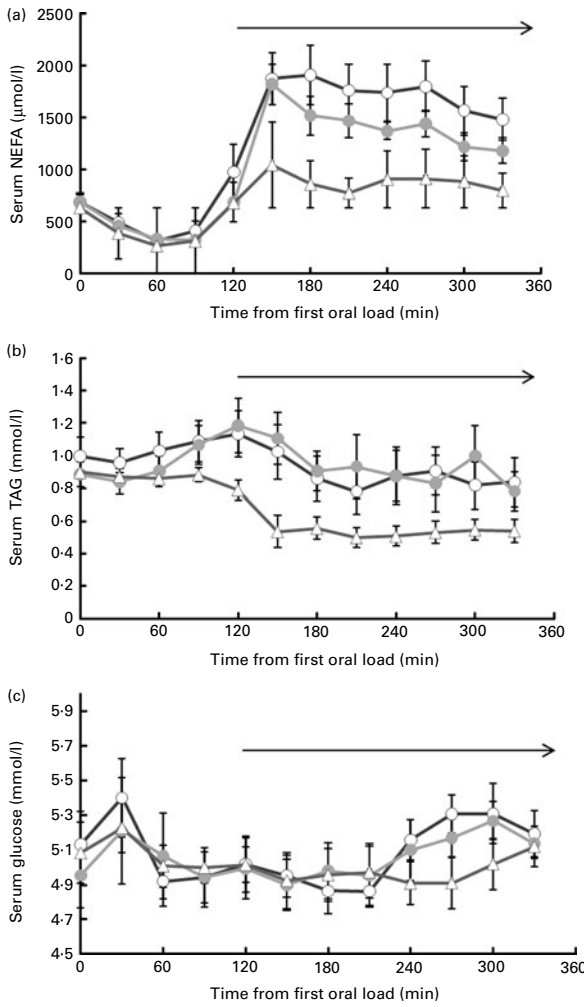
Fig. 1. Serum (a) NEFA, (b) TAG and (c) glucose (note the broken scale of the y-axis) during the consumption of SFA (○), MUFA (●) or control, no fat (△). Values are means of ten subjects, with their standard errors represented by vertical bars; ⟶ indicates administration of heparin. For serum NEFA and TAG, repeated-measures ANOVA revealed a significant effect of test drink ($P < 0.001$), time ($P < 0.001$) and test drink × time interaction (NEFA $P = 0.031$, TAG $P < 0.001$). For glucose, there was a significant effect of time only ($P < 0.001$).

Serum insulin, C-peptide and gastric inhibitory peptide response

Serum insulin levels increased following the bolus oral load of both fats, reaching a peak at 60 min (Fig. 2(a)). There was no insulin response to consumption of the control drink. Following administration of heparin, there was a sharp increase in insulin for the MUFA-rich drink at 150 min (95 % CI 43·3, 65·3 pmol/l), which was significantly different from the control at this time point (95 % CI 14·4, 36·9 pmol/l). In contrast, there was no rise in serum insulin at 150 min for the SFA-rich drink (95 % CI 27·1, 49 pmol/l). There was a significant effect of test drink, time and test drink × time interaction for overall insulin response ($P < 0.001$), with both SFA- and MUFA-rich drinks eliciting a higher insulin response than the control drink ($P < 0.001$); however, there was no significant difference

Table 2. Fatty acid composition (percentage weight of total fatty acids) of NEFA and TAG during the study days*

(Mean values with their standard errors for four subjects)

	NEFA									TAG								
	Baseline		SFA		MUFA		Control		ANOVA	Baseline		SFA		MUFA		Control		ANOVA
Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P
Palmitic	23·8	1·0	37·9†‡	2·0	14·7†§	0·5	23·9	0·8	<0·001	30·8	3·6	P	1·6	12·1†	0·4	26·1	1·7	<0·001
Stearic	23·7	2·0	13·8	0·6	14·1	0·8	19·5	1·9	0·001	5·4	1·5	P	0·3	3·7	0·2	4·3	0·3	0·381
Oleic	19·5	3·1	28·4‡	0·7	47·9†§	1·8	24·4	2·3	0·001	42·9	4·1	P	1·0	72·1†§	0·6	44·7	1·6	<0·001
Linoleic	6·2	0·6	8·1	0·5	7·0	0·3	8·9†	0·5	0·001	13·3	2·9	P	1·1	9·9§	1·0	16·4	1·3	0·020

* Baseline composition was measured from serum samples collected at 0 min (pooled from all three study days), while samples collected between 300 and 360 min were used to determine composition during consumption of the different test drinks.

† Mean values were significantly different from that of baseline ($P < 0.05$).

‡ Mean values were significantly different from that of MUFA ($P < 0.05$).

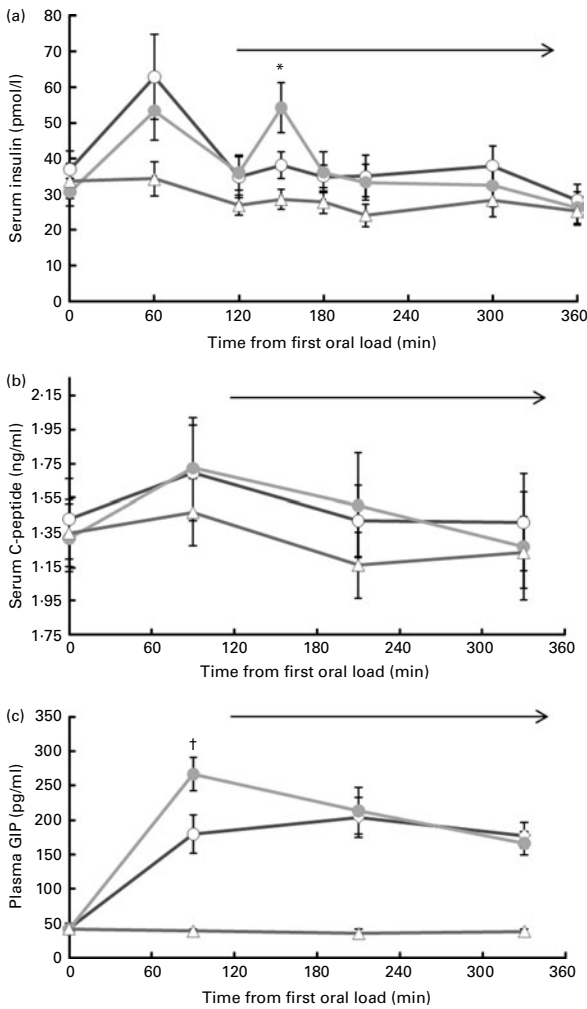§ Mean values were significantly different from that of control ($P < 0.05$).

Fig. 2. Serum (a) insulin, (b) C-peptide (note the broken scale of the y-axis) and (c) gastric inhibitory peptide (GIP) during the consumption of SFA (○), MUFA (●) or control, no fat (△). Values are means of ten, seven and nine subjects, respectively, with standard errors represented by vertical bars; ⟶ indicates administration of heparin. *Denotes a significantly higher response for MUFA than control; †denotes a significantly higher response for MUFA than for SFA ($P < 0.05$). For insulin and GIP, repeated-measures ANOVA revealed a significant effect of test drink, time and test drink × time interaction ($P < 0.001$). A significant effect of test drink ($P = 0.010$) and time ($P < 0.001$) was observed for C-peptide.

between the fats ($P = 1.000$). For C-peptide, there was a significant effect of test drink ($P = 0.010$) and time ($P < 0.001$) (Fig. 2(b)). Compared with the control drink, the response was significantly higher for the SFA-rich drink ($P = 0.013$), with also a strong trend for MUFA values to be higher than the control ($P = 0.056$), but there was no difference between the fats ($P = 1.000$).

There was a marked increase in plasma GIP following the consumption of both fat-containing drinks, which was sustained for the duration of the study day (Fig. 2(c)). Repeated-measures ANOVA revealed a significant effect of test drink, time and test drink × time interaction ($P < 0.001$); both fats induced a higher response than the control ($P < 0.001$). Despite no statistically significant overall difference between the fats ($P = 0.097$), MUFA induced a

significantly higher early response at 90 min (95 % CI 232, 302 pg/ml) than SFA (95 % CI 145, 215 pg/ml).

Markers of endothelial function

Despite the high inter-subject variation in SI_{DVP} during the study days (Fig. 3), there was a clear increase in this measure during the consumption of the SFA-rich drink, which peaked at 210 min. In the case of the MUFA-rich drink, values declined towards 210 min, increasing slightly at 330 min. Because of varied baselines between the study days, SI_{DVP} was analysed by a repeated-measures ANCOVA using mean SI_{DVP} (90–330 min) as the dependent variable; this revealed a significant effect of test drink ($P = 0.001$). SI_{DVP} was higher during the consumption of the SFA-rich drink than during the consumption of the control drink ($P < 0.001$), but there was no statistical significance between the MUFA-rich and control drinks ($P = 0.089$) or the SFA-rich and MUFA drinks ($P = 0.128$).

Table 3 shows the effect of test drinks on circulating markers of endothelial function. There was a significant effect of test drink on sI-CAM levels ($P = 0.014$). Pairwise analysis showed that levels of sI-CAM were significantly higher during the consumption of the SFA-rich drink than during the consumption of either the MUFA-rich ($P = 0.048$) or the control ($P = 0.021$) drink, while there was no difference between the response to MUFA and the control ($P = 1.000$). PAI-1 and NO_x decreased during the study days, while ET-1 increased ($P < 0.001$), but there was no effect of test drink compared with the control for any of these parameters.

Discussion

The present study demonstrates that acute elevation of serum NEFA by the consumption of a SFA-rich drink leads to an increase in arterial stiffness and levels of sI-CAM in healthy young men. These effects were not found following ingestion of a MUFA-rich drink, suggesting that the composition of fat consumed is an important factor in determining the impact of elevated NEFA on both vascular function and endothelial inflammation. The present protocol resulted in a three- to
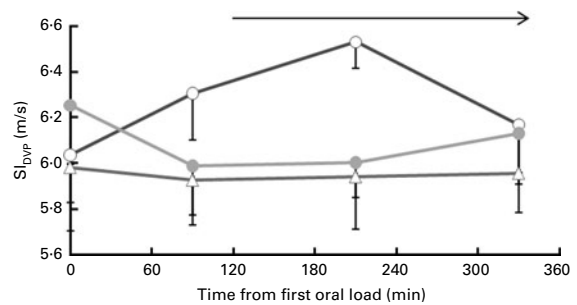

Fig. 3. Arterial stiffness index during the consumption of SFA (○), MUFA (●) or control, no fat (△) (note the broken scale of the y-axis). Values are means of eight subjects, with their standard errors represented by vertical bars; ⟶ indicates administration of heparin. Repeated-measures ANCOVA of mean stiffness index derived from digital volume pulse (90–330 min) revealed a significant effect of test drink ($P = 0.001$).

Table 3. Circulating markers of endothelial function during the study days

(Mean values with their standard errors, n 10)

	SFA				MUFA				Control				ANCOVA
	0 min		330 min		0 min		330 min		0 min		330 min		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P
sI-CAM (ng/ml)	178	18	184*	20	174	16	168†	16	168	17	166	17	0·014
PAI-1 (ng/ml)	4·7	0·9	2·6	0·56	3·4	0·58	2·2	0·51	4·5	0·97	3·1	0·57	0·346
ET-1 (ng/ml)	1·16	0·06	1·24	0·07	1·22	0·05	1·24	0·07	1·20	0·08	1·20	0·04	0·815
NO$_x$ (μM)	13·4	2·1	9·2	1·3	10·9	1·4	8·7	1·1	11·2	1·4	7·1	1·1	0·127

sI-CAM, soluble intercellular cell adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; ET-1, endothelin-1; NO$_x$, total nitrites.
* Mean value was significantly different from that of control ($P < 0.05$).
† Mean value was significantly different from that of SFA ($P < 0.05$).

fourfold increase in NEFA from baseline in our healthy subjects, achieving levels observed in insulin-resistant states such as obesity and T2D. A subset analysis of fatty acid composition of NEFA and TAG also confirmed that the protocol significantly increased the proportions of palmitic and oleic acids during the SFA and MUFA study days, respectively.

The present study confirms the findings of published lipid infusion studies, which have shown that experimental acute elevation of NEFA impairs vascular function[4,30–36]; this is also reflected in postprandial studies, which have shown similar adverse effects of all types of high-fat meals[37]. However, unlike the findings of lipid infusion studies, which have analysed markers of vascular function and inflammation in response to elevating NEFA rich in *n*-6 PUFA, the protocol adopted in the present study has enabled a comparison of the acute effects of SFA and MUFA. As such, there are no previous studies to directly compare the present results, although several postprandial studies have compared the effect of mixed meals rich in SFA or MUFA on vascular function, with variable results. Comparison of outcomes from different postprandial studies is complicated by virtue of differences in the amount and source of the fats used in the oral fat load. For example, shea butter is rich in stearic acid and has been shown to have varying absorption rates following acute ingestion, which would confound data from this type of study[38]. In addition, the amount and type of carbohydrate in test meals in different studies is also variable which, through differing effects on insulin secretion, would introduce additional variables other than the fat type. Raitakari *et al.*[14] reported that FMD did not change after a cooked breakfast rich in SFA or MUFA. Rueda-Clausen *et al.*[13] also found a similar reduction in FMD after consumption of potato soup containing either olive oil or palm oil. In contrast, Berry *et al.*[15] found a significant reduction in FMD after a meal of muffins and a milkshake enriched in high-oleic sunflower oil, which was not found when the meal was enriched with shea butter. However, in this latter study, consumption of the MUFA-rich meal led to significantly higher circulating TAG levels than consumption of the SFA-rich meal. This could affect vascular function as the extent of postprandial lipaemia has been shown to be negatively correlated with FMD in healthy subjects consuming high-fat meals[39,40]. In the present study, the oils used for the SFA fat load were chosen to reflect

typical UK fatty acid intakes, and, importantly, no difference was recorded between circulating TAG levels during consumption of either the SFA- or the MUFA-rich fat loads, suggesting that the observed differences in vascular markers are due to qualitative rather than quantitative differences in the raised serum NEFA. Since circulating TAG-rich lipoproteins have been shown to interact directly with the endothelium, the possibility that differences in the composition of TAG in these particles may be responsible for the observed effects on endothelial function should also be considered.

One mechanism linking fatty acids to vascular function is the potential effect on insulin secretion, as insulin is an important mediator of cardiovascular health[41]. Consumption of both fat loads caused a prompt increase in serum insulin peaking at 60 min, with a concomitant increase in serum C-peptide. Both saturated and unsaturated NEFA have been shown to act directly on pancreatic β-cells to acutely increase insulin secretion *in vitro*; however, *in vivo*, the impact of NEFA on insulin depends on the prevailing glucose concentration, with a limited effect of NEFA at low glucose concentrations[42]. Although the glucose load in the drinks was minimal, a small increase in blood glucose concentration was observed at 30 min for all drinks, including the control, which suggests that the early insulin peak observed for the two fat-containing drinks reflects glucose-stimulated insulin secretion. Enhanced insulin secretion at 60 min observed for the fat-containing drinks, despite similar increases in glucose at 30 min for all three drinks, illustrates the potentiating effect of oral fatty acids on glucose-stimulated insulin secretion and influence of fat ingestion on glucose homeostasis[43]. It has long been known that oral fat is the most potent stimulus to GIP secretion. *In vitro* studies have shown that fatty acids can directly influence the release of GIP from the K-cells of the intestine, and that GIP binds to specific G-protein-linked receptors on the surface of the pancreatic β-cells to stimulate insulin secretion[42]. Although the overall GIP responses were similar for the two fats, there was a higher GIP concentration at 90 min for the MUFA- *v.* the SFA-rich fat load, which may have contributed to the second insulin response at 150 min for the MUFA fat load, which was not observed for the SFA fat load. The findings in the present study are consistent with those of Thomsen *et al.*[20], who reported a greater increase in GIP and GLP-1 after consumption of a mixed

meal containing olive oil *v.* butter, as well as those of Brynes *et al.*[44] who reported a trend towards greater GLP-1 levels after consumption of olive oil *v.* butter or maize oil. In contrast, Zampelas *et al.*[45] found no differences in GIP response after consumption of mixed meals rich in either SFA, *n*-6 PUFA or *n*-3 PUFA. We have no direct evidence to link the more adverse effect of a SFA- than a MUFA-rich fat load on arterial stiffness to the differences that we have observed in circulating incretin or insulin, but the protocol that we have used does add further support for an acute impact of fatty acid composition on the entero-insular axis.

Previous studies of the effect of dietary fat on markers of endothelial dysfunction have produced inconsistent findings. Nappo *et al.*[46] found that consumption of a high-fat meal increased circulating sI-CAM levels, but Dekker *et al.*[47] found no effect of a series of high-fat meals varying in their polyunsaturated:saturated ratio on either sI-CAM or soluble vascular adhesion molecule-1 levels. The present study found that acute elevation of NEFA during consumption of a SFA-rich but not of a MUFA-rich fat load resulted in modestly increased levels of sI-CAM, a circulating indicator of endothelial inflammation, which has been proposed as a useful marker of underlying coronary artery disease in healthy subjects[48]. Although there are a number of mechanisms whereby elevation of NEFA may be linked with endothelial inflammation and dysfunction[32,48,49], an increase in sI-CAM may indicate imbalance in insulin signalling in the endothelium since stimulation of the mitogen-activated protein kinase pathway, but not of the phosphoinositide 3-kinase pathway, results in an increase in the expression of cellular adhesion molecules, including sI-CAM. This may have a significant impact on endothelial function, as insulin signalling is a mediator of vascular tone through the generation of NO via the phosphoinositide 3-kinase pathway and the vasoconstrictor ET-1 via the mitogen-activated protein kinase pathway; this latter pathway can also lead to the generation of pro-thrombotic PAI-1[41]. However, the present study found no difference in the response to the different test drinks of circulating markers of endothelium function (ET-1, NO_x and PAI-1) other than the modest increase that we have observed in sI-CAM. There is therefore insufficient evidence to conclude that the increase in arterial stiffness reported with the SFA-rich fat load is related to the impairment of insulin signalling pathways that regulate NO release or the expression of these proteins.

The protocol used in the present study achieved a successful threefold elevation of circulating NEFA, comparable with that found in the study of Beysen *et al.*[26]. Subjects in the present study had higher fasting levels of NEFA (average 667 μmol/l) compared with the group of Beysen *et al.* (260–400 μmol/l), which may have contributed to the higher absolute NEFA values that we observed following heparin administration. A limitation of our sample processing meant that up to 30 min could have elapsed before samples were stored at 4°C; this could have resulted in a degree of *in vitro* lipolysis in the post-heparin samples and may have contributed to the higher values that we have reported here.

In conclusion, the main finding of the present study is that an acute elevation of serum NEFA following consumption of a SFA-rich drink has an adverse impact on arterial stiffness and sI-CAM levels. The study also confirms that ingestion of a fat load leads to sustained increases in the incretin hormone GIP, with some evidence for a more marked effect of MUFA than for SFA. It is not possible to determine whether the effects of different fats on vascular tone and endothelial inflammation are related to differential effects on incretin or insulin secretion and/or action at the endothelium. However, given the known effects of NEFA elevation on other insulin-sensitive tissues such as skeletal muscle and adipose tissue, further elucidation of the effects of fat type on the sensitivity of the endothelium to insulin is warranted.

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